ELECTRONIC NOSE DISCRIMINATION, ANALYTICAL IDENTIFICATION, AND SENSORY CONFIRMATION OF FLAVOR IMPACT COMPONENTS IN CITRUS JUICES

By

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Abstract of Thesis Presented to the Graduate School
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ELECTRONIC NOSE DISCRIMINATION, ANALYTICAL IDENTIFICATION, AND SENSORY CONFIRMATION OF FLAVOR IMPACT COMPONENTS IN CITRUS JUICES

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Orange and grapefruit juices were evaluated for flavor characteristics using sensory techniques and compared with results from three different electronic noses. Volatile components were extracted from juices using both solid phase microextraction (SPME) and liquid-liquid extraction. Volatiles were characterized by gas chromatography-olfactometry (GCO) and identified by GC and mass spectrometry (MS).

A 12 metal oxide sensor ENose utilizing multivariate statistical analysis (MVSA) could distinguish between 26 different grapefruit juice cultivars. A 12 organic polymer sensor ENose exhibited some success in discriminating between orange juices (OJ) exposed to different heat treatments (unpasteurized, heated to 96° C for: 8 seconds, 120 seconds, and in excess of 180 seconds) but was less successful in discriminating between OJ from different cultivars. A 32 organic polymer sensor array ENose utilizing MVSA could differentiate between unpasteurized OJ (UPOJ) stored for 0, 3, 6, 9, and 12 days at 2° C. Results from Canonical discriminant analysis of ENose data were similar to that from sensory panel, and from headspace GC data from the identical UPOJ stored for different times.

Headspace aroma components from UPOJ and heat abused early-mid season OJ were extracted using SPME and analyzed using GCO. Eighteen aroma peaks were common to both heat treated and UPOJ. Six aroma active peaks were observed in only UPOJ headspace. Five different aroma components were observed in heat abused juice. GCO analysis of liquid-liquid extracts from unpasteurized and severely heated Valencia juice found 23 aroma peaks common to both samples, but found 17 peaks only in UPOJ, and 2 peaks only in the heat abused sample.

Recombination of 8 intense aroma contributors of UPOJ, plus 4 additional components reported in OJ, added to model juice solutions did not differ significantly ($p<.05$) in orange aroma and flavor from an UPOJ previously frozen.

The primary source of linalool, a major flavor impact compound, in Valencia orange juice was from the peel oil which is typically introduced during juice extraction. Highest concentrations of linalool were found in juices which experienced the most peel maceration during extraction.

CHAPTER 1

INTRODUCTION

In 1998, citrus was worth an estimated $1,015,703,000 in the state of Florida. Oranges were the most important citrus fruit with an estimated value of $876,042,000 or 86 % of the total Florida citrus market (Geuder et al., 1998). It is ironic, given orange juices' enormous economic value, the small number of published studies conducted that have addressed orange juice aroma volatiles, their importance in juice quality, and then followed up with sensory studies that sought to confirm analytical findings. This research was conducted to determine the effectiveness of "electronic nose" instrumentation in differentiating citrus juices based on headspace components, to identify aroma active volatiles responsible for fresh and heat abused orange juice flavors, and to determine the source of linalool in Valencia orange juice and the effect of heat treatment on linalool concentration.

Electronic Nose

Sensory analysis has been the historical method of choice for measuring flavor differences between food samples produced by different processing conditions. At a minimum, sensory analysis requires several panelists for statistically significant results, and additional time to set up, conduct, and statistically evaluate data. If descriptive analysis

(DA) is utilized, sensory standards must be prepared and the sensory panel must be trained over several weeks time. Most descriptive panel training programs demand between 40 and 120 hours of training (Meilgaard et al., 1991). The term "electronic nose" (ENose) describes an emerging technology which can dramatically shorten time required to determine if differences exist between products (Hodgins and Conover, 1995). Although several ENose instruments are available and each has unique attributes (sample temperature controls, method of headspace sampling, calibration techniques), all such instruments have sensors that interact with volatiles and provide a different output that can be used as a means to distinguish between samples. Current instrument designs employ organic polymer or metal oxide based sensors. Signals generated from sensor-volatile interaction are imported into a spreadsheet and analyzed with multivariate statistics (typically Principal Components Analysis or Artificial Neural Networks). ENoses were utilized to measure differences between grapefruit juice varieties, between grapefruit juice and orange juice, and between orange juices exposed to different heat treatments and varying storage times.

Gas Chromatography-Olfactometry

A cornerstone technique for identifying and characterizing aroma active components is gas chromatography-olfactometry (GCO). GCO combines sensory analysis and chromatographic separation science to identify which peaks eluting from a GC column exhibit aroma activity. The method employed in this dissertation was Osme, a sensory time-intensity technique. As a peak with aroma activity elutes, panelists rate the intensity of the aroma, verbally describe the aroma using a sliding lever whose position is recorded via computer (Miranda-Lopez et al., 1992). A major advantage of Osme over dilution techniques is that it can generate an aromagram (plot of aroma intensity vs. time) after a single run. GCO techniques such as CHARM and Aroma Extraction Dilution Analysis (AEDA) require 3-9 runs of successively diluted sample until no aroma active components are detected. Dilution analysis permits aroma intensities to be evaluated based on its experimentally determined aroma threshold. There have been no published studies using Osme to investigate orange juice volatiles. CHARM analysis was utilized by Marin et al.(1992) to determine the effect of plastic packing polymers on orange juice volatiles. Details of this study are discussed in Chapter 4. More detailed literature reviews are found in the introductions of individual chapters. AEDA was utilized to investigate important volatiles in unpasteurized Valencia orange juice in a study conducted by Hinterholzer and Schieberle (1998).

Sensory Studies

Orange juice studies utilizing GC and sensory analysis have been conducted more often than orange juice studies utilizing GCO. Among these the most important include: Ahmed et al.(1978b) identified components that contribute to fresh orange flavor, Tamura et al. (1996) compiled a list of components important to Navel orange flavor, Tatum et al. (1975) identified 3 components implicated in cooked orange juice flavor.

Volatile Extraction

Extraction method had an important effect on sensory and analytical results including GCO aromagrams and GC FID chromatograms. In this study two types of extraction methods were utilized, Solid Phase Microextraction (SPME) and liquid-liquid extraction (pentane:diethyl ether). Each technique has advantages. SPME is a solventless technique that can extract early eluting, low molecular weight components such as ethanol and acetaldehyde that would be obscured by the large solvent peak of pentane:diethyl ether. SPME is highly selective for terpenes. In contrast, pentane:diethyl ether extractions were more reproducible, and extracted a wider range of volatile components.

Linalool

Linalool, a terpene alcohol, is an aroma active component important in a number of beverages and fruit products including citrus (Clark and Chamblee, 1992). Marin et al. (1992) reported linalool was the most aroma active component in pasteurized, mechanically squeezed orange juice. The aroma activity was less in hand squeezed, unpasteurized juice. Source of linalool in Valencia orange juice and effect of heat treatment on linalool concentration was examined in Chapter 7.

Summary

Citrus flavor research presented in this dissertation investigated product discrimination (sensory panel and ENose), extraction methodology (SPME vs. pentane:diethyl ether), identification of important aroma active components (GCO, GC, and mass spectrometry), and correlation with sensory analysis.

CHAPTER 2

DISCRIMINATION OF GRAPEFRUIT JUICE VARIETAL DIFFERENCES BY AN ELECTRONIC NOSE EQUIPPED WITH METAL OXIDE SENSORS

Introduction

In the State of Florida, agriculture is second only to tourism in industrial size and capital value. Grapefruit for the 1995-96 season was worth an estimated $102,639,000; second only to orange as the largest agricultural product from Florida (Terry et al., 1996). The majority of grapefruit is processed for juice rather than fresh fruit. New cultivars of grapefruit have been developed primarily to increase red pigmentation and to reduce the number of seeds per fruit. Breeding programs are currently focusing on increasing fruit yield (per acre), extending the maturation season, and increasing disease and parasite resistance. The characteristic flavor of a grapefruit cultivar is influenced primarily by genetics, however other factors which are important contributors include tree position, maturity, climate, rootstock and seasonal variation.

The primary objective of this study was to determine if 24 grapefruit cultivars (Table 2-1) could be differentiated on the basis of their volatile composition using an electronic nose equipped with metal oxide sensors. Although grapefruit flavor is influenced by both aroma (volatiles) and taste (non-volatiles) components, a secondary objective of this study was to determine if there existed a relationship between overall acceptability of grapefruit juice flavor and signals induced from volatiles in metal oxide sensors. Grapefruit may also be classified as either red, pink or white based on the amount of lycopene and beta carotene (red/orange pigment) in the flesh and juice. Lycopene and beta carotene are carotenoids, and carotenoids have been shown to degrade and produce potent aroma active compounds (Winterhalter, 1992).

Table 2-1 Grapefruit varieties examined

Cultivar	**color**	**Average hedonic score***
Florida red	red	9
Inman late	White	8
Marsh 10b-5-7	White	8
Mott	White	8
Pink Marsh	red	8
Ray Ruby	red	8
Shambar	red	8
Star Ruby	red	8
Alsop	red	8
Duncan	White	8
Foster	red	8
Henderson	red	8
Hybrid 1-42-115	White	7
Marsh 9E-5-22	White	7
Red Blush	red	7
Davis	White	7
Hybrid 1-82-19	White	6
Navel	White	6
Hudson	red	5
Hybrid 1-81-57	White	5
Conner	White	5
Hybrid 1-43-5	White	4
Hybrid 1-77-19	White	4
Natsudaidai	White	1

*Hedonic scores were from initial sensory screening.

Therefore a secondary objective of this study was to determine if the presence of these carotenoid pigments would alter the aroma profile of the juice compared to non-pigmented (white) grapefruit juice.

Both volatile and non-volatile components are important contributors to grapefruit juice flavor. Non-volatile components important in grapefruit juice flavor include sucrose, glucose and fructose, the sugars which impart sweetness. Additionally, citric acid imparts the sour, tart flavor. Flavanone glycosides (neohesperidosides) and limonoids are responsible for the characteristic bitter flavor of grapefruit. The relationship between bitterness and juice acceptability has been the topic of numerous investigations (Shaw et al., 1984; Dougherty et al., 1977; Burkhardt, 1971). Studies conducted to quantify and remove bitter components are even more numerous (Shaw et al., 1991; Wethern, 1991; Van-Beek and Blaakmeer, 1989; Rouseff, 1988).

Volatile components in grapefruit originate in the flavedo (outer section of the peel), and in juice sacs. While components in the peel oil are almost exclusively oil soluble, volatiles in the juice sacs may be oil or water soluble. This is due to the presence of small amounts of oil in juice sacs that differs slightly in composition from the oil present in the peel (Rouseff et al., 1994). The identification of grapefruit volatiles has been investigated by Shaw and Wilson (1980). Important aroma active volatile components commonly found in grapefruit include short-chain alcohols, esters, hydrocarbons, thiols and aldehydes (Pino et al., 1986).

Materials and Methods

Sample Preparation

Grapefruit were harvested 4 June 1997 from the United States Department of Agriculture's Whitmore Foundation Farm in central Florida. Six to eight fruit were harvested from each tree. Fruit were juiced within 2 days using a Sunkist single head mechanical reamer. Juice was promptly placed in 1L amber screw cap glass bottles and stored at - 20° C. On the day of examination, frozen juice was quickly thawed by placing the bottle under a 30° C stream of water. Volatile analyses were conducted with an Alpha M.O.S. model 3000 (Alpha M.O.S. America, Belle Mead, NJ) equipped with 12 metal oxide sensors. Twenty mL of juice was placed in a 135 mL glass vessel supplied with the instrument. Samples were maintained at 25 °C. Headspace humidity was maintained at 30% by saturating a double distilled water solution with calcium chloride and heating the solution to 40° C. Oxygen was utilized to flush the headspace and sensor surface. Headspace generation, sampling, purge and delay times were 60 seconds, 200 seconds, 200 seconds and 300 seconds, respectively.

Sensory Analysis

In order to determine if patterns discerned from statistical analysis of the metal oxide sensor responses could be correlated with flavor, sensory panel screening of grapefruit varieties was conducted. Four experienced panelists rated juice acceptability on a 1 to 9 point hedonic scale, with 9 indicating highly acceptable and 1 strongly unacceptable.

Electronic Nose Analysis

Each juice was analyzed in the form of two separate samples, each analyzed three times. Analysis of three of the juices rated most acceptable and the three rated least acceptable were repeated to produce 15 additional (5 samples x 3) values as a separate set.

Statistical Analysis

Sensor data were imported into Statistica (StatSoft, Tulsa, OK; version 5). Canonical discriminant analysis was utilized to determine if signals from sensors could be grouped in accordance with specified variables such as fruit varieties and hedonic groups (Garner et. al, 1992). When groups consisted of two variables, a statistical method was employed to determine the distances between the grouping variable center (mean) and cultivar responses. The method utilized, Mahalanobis distances, also allowed for prediction to which group a cultivar belonged based on distances to group centroids. The success of correctly classifying grapefruit varieties into the categories acceptable versus not acceptable and as red pigmented versus white pigmented was determined with this procedure.

Results and Discussion

For each assay the Fox 3000 software selects the maximum response of time-conductance voltage data generated by sensors. The maximum signal is used for statistical analysis which should allow for sample distinction. Canonical discriminant analysis (grouping variable = cultivar type) of data from assay of each grapefruit juice is shown in Figure 2-1 with the two sets of triplicate analyses of Florida Red and Natsudaidai cultivars indicated. Within sample replication (3 replicates, 2 samples for each cultivar) was better than between sample replication as evidenced by the tighter groupings for each sample. Results indicated most cultivars were grouped similarly, thus replication was good with a percentage relative standard deviation between runs at generally less than 5%. Although samples from individual cultivars replicated well, inferences about volatile composition and flavor between different cultivars were difficult to summarize due to the large size of the data set.

Sensory Screening

To determine if sensor signals from these samples could be grouped in a manner consistent with overall cultivar acceptability, sensory screening was conducted. Screening revealed grapefruit juice flavor varied substantially between cultivars. Taste varied from sweet and slightly tart, to overwhelmingly acidic and bitter. Aroma ranged from fruity and floral to sulfury and skunky. Screening results were divided into three categories for discriminant analysis grouping variables: unacceptable scores 1-4, slightly acceptable scores 5-7, and highly acceptable scores 8-9. Canonical discriminant analysis of sensor response with assigned grouping variable showed response could be separated into groups that correlated with juice flavor acceptability (Figure 2-2). The overlap observed between juice categories appeared to be due to non-volatile components of flavor as seen in Figure 2-2. For example hybrid 1-43-5 was very sour (acidic), but the volatiles were apparently similar to some of the most preferred juices. Sensory screening determined three of the most acceptable juices were from Pink Marsh, Ray Ruby, and Inman Late cultivars.

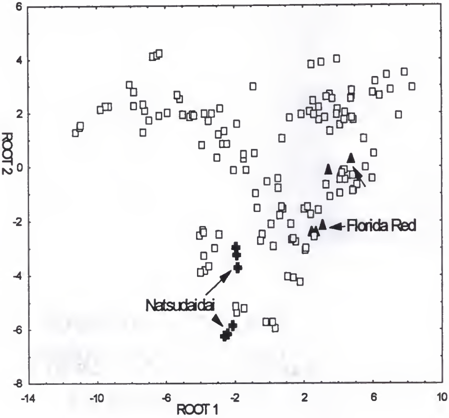


Figure 2-1 Discriminant analysis of metal oxide sensor response to 24 varieties of grapefruit juice with varieties Florida Red and Natsudaidai indicated. Each sample replicated 6 times.

These juices possessed a pleasing balance of sugar and acid plus they were very fruity. The three least acceptable juices were Natsudaidai, hybrid 1-77-19, and hybrid 1-43-5. These juices were extremely acidic, unusual for late season grapefruit.

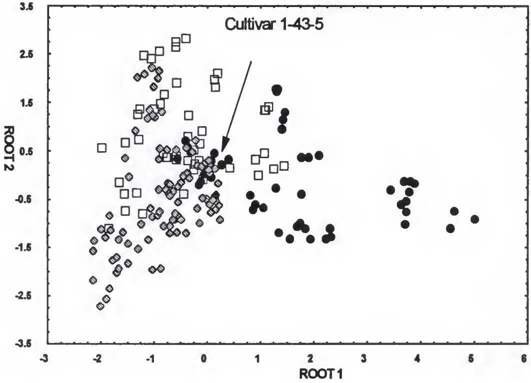


Figure 2-2 Discriminant analysis of grapefruit juice sensor response grouped as hedonic categories with (●) indicating scores 1-4, (□) indicating scores 5-7, and (◈) indicating scores 8 and 9.

Discriminant analysis of sensor signals from these 6 cultivars with grouping variables high preference and low preference revealed signals could be grouped as most acceptable and least acceptable juices with an 81% accuracy. Misclassified cultivars include 1-43-5. As previously mentioned, this was due to the unpleasant taste (excessive sourness) of the juice. The Mahalanobis distances of the sensor responses from nine of fifteen replicates of this cultivar indicated it belonged to the highly acceptable group. Pink Marsh grapefruit, one of the cultivars in the highly preferred category, was also misclassified four of fifteen times.

Data from the sensor responses of three of the most acceptable and the three least acceptable cultivars were re-evaluated, but instead of using high and low preference as the 2 grouping variables, each of the six individual cultivars were used as a separate category. As shown in Figure 2-3, all six cultivars were separated with no overlap. Interestingly, the three highly preferred juices were tightly clustered suggesting that higher preference juices have similar volatiles. The three least preferred juices were widely separated suggesting large differences in volatile composition. Thus, there may be several different components in the poorly preferred juices which cause them to be downgraded.

Fruit Pigmentation and Sensor Response

To determine if fruit color (pigmentation) is correlated with volatile composition and sensor response, grapefruit juices were assigned to either a red or white group, based on the presence or absence of pigment color. Correct assignment of all samples was 69%, but only 44% of red varieties were properly classified. For white, 12% were misclassified

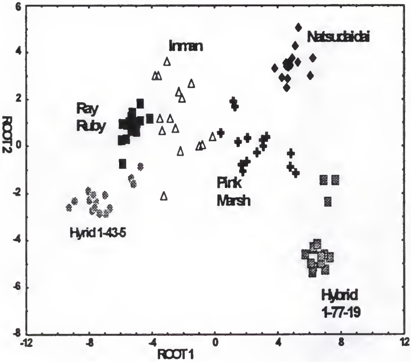


Figure 2-3 Discriminant analysis of sensor response to 3 of the most favored and the 3 least favored grapefruit juices grouped as individual cultivar.

as red. It was concluded there was some correlation between sensor response due to a fruit's volatile composition and it's color.

Study Implications

Our results suggest that the electronic nose could be used for grapefruit juice quality control. An instrument could distinguish between the most acceptable and the least acceptable juices in a reasonably rapid, consistent manner.

Correlation with Other Studies

We have shown that volatiles from the juices of different cultivars of grapefruit can be evaluated through discriminant analysis of metal oxide sensor signals. Responses from juices of various cultivars form tight groups which can easily be distinguished from one another (Figure 2-3). Other researchers have successfully used electronic nose technology to discriminate between different food products. Gardner et al. (1992) discriminated between commercial coffees and also between coffees roasted for varying times using 12 tin oxide sensors. They employed discriminant analysis to determine the accuracies of their category predictions. They also reported tin oxide sensors are particularly sensitive to thermally generated volatile components. For this reason, they were more successful at discriminating between coffee roasted for different times (88.1% of the samples were correctly classified) than between blends (80% correctly classified). Simon et al. (1996) measured blueberry quality and fruit ripeness with an instrument developed at Purdue University that utilized 2 tin oxide sensors. As a fruit matured the quantity of volatiles produced increased which cause an increase in sensor response. Thus, they could estimate blueberry ripeness from the corresponding sensor responses.

Future Studies

Additional information on maturity effects of volatiles important in distinguishing between cultivars, and distinguishing between acceptable and unacceptable juices is needed. Analysis by descriptive panel could specify what flavor attributes are most important in distinguishing between juices. Identification of volatiles responsible for these attributes and sensitivity of sensors to these components are areas for future work.

CHAPTER 3

DISCRIMINATION OF THERMALLY TREATED ORANGE JUICES BY AN ELECTRONIC NOSE EQUIPPED WITH ORGANIC POLYMER SENSORS

Introduction

The most important orange cultivars processed for juice in the United States are Valencia, Hamlin, Pineapple, and Parson Brown. The harvesting season begins in October and ends in late spring. Hamlin, Pineapple and Parson Brown oranges mature in the early to mid season. Commercially, they are processed together so their juices are normally mixed. Valencia matures in late season and is processed separately from the early-mid cultivars.

Degradation and shortening of orange juice shelf-life is due primarily to unwanted enzymatic activity and spoilage microorganisms. Pectin, a major constituent of orange juice pulp, is a polysaccharide composed of linked D-galacturonic acid monomers with periodically substituted methoxyl groups as well as occasional L-arabinose, D-galactose, and D-xylose units mixed in the polymeric chain. Pectin methylesterase, an enzyme naturally present in oranges, facilitates the cleavage of methyl esters from pectin. The resultant anions formed from 2 polymers may then form ionic bonds with free calcium ions. These adjoined polymers precipitate and the result is loss of juice "cloud," a significant problem in juice quality. *Lactobacillus* and *Leuconostoc* genera are 2 spoilage bacteria commonly found in orange juice. *Saccharomyces cerevisiae*, the versatile yeast involved in the production of beer, wine, and bread leavening, also can produce ethanol in orange juice. Pectin methylesterase, *Lactobacillus, Leuconostoc*, and *Saccharomyces cereviae* are prime candidates for pasteurization destruction.

Heat treatment is the most common form of pasteurization employed by the food industry to destroy dangerous microorganisms and prolong the shelf-life of liquid food products. Heat treatment may also cause unwanted off flavors and significantly diminish orange juice flavor quality if the process is too hot or too long (Fellers and Carter, 1993b; Tocchini et al., 1984; Schachter and Fedele, 1982).

Identification of Orange Juice Components

The determination of major chemical components present in orange juice has been the subject of numerous investigations for almost 40 years (Moshonas and Shaw, 1992; Paik and Venables, 1991b; Nisperos and Shaw, 1990; Dinsmore and Nagy, 1971).

Aroma Activities of Orange Juice Components

Volatile components responsible for fresh orange aroma and flavor were investigated by Ahmed et al. (1978a). A mixture of acetaldehyde, citral, ethyl butanoate, d-limonene, and octanal favorably influenced the aroma and flavor of juice. Decanal, trans-2-hexanal, and citronellal had a negative influence. Nelson and Tressler (1980) found acetaldehyde and ethyl butanoate positively contributed to flavor whereas trans-2-hexenal and penten-3-one were detrimental. Moshonas and Shaw (1994) reported 24 of

46 quantified components in 13 separate fresh orange juice samples had levels of aroma active volatiles above the threshold values. The techniques for determining sensory characteristics of juices vary. The primary objective of this study was to determine if an electronic nose equipped with organic polymer sensors could distinguish between orange juices subjected to different time-temperature treatments ranging from none to extreme. If successful, such an instrument would be a major asset to citrus processors.

Materials and Methods

Mature early-mid season oranges (Hamlin, Pineapple, and Parson Brown cultivars) were harvested from four central Florida groves on 15 and 16 January 1997. Mature late season (Valencia) oranges were harvested 14 May 1997 from a central Florida grove. Following harvesting, fruit was immediately shipped to Sun Pure corporation (Sun Pure; Avon Park, FL) where Brown extractors were utilized for juicing.

Heat Treatment

Early-mid season juice were processed on 17 January 1997 and late season on 15 May 1997. The two types of juice were divided into four samples each. One sample (unpasteurized) was immediately stored in 1L amber glass bottles with Teflon screw caps at -20 ° C. The other three samples were pasteurized with a Microthermic UHT / HTST pasteurizing system, model 35 (Microthermic Corp, Raleigh, NC). Flow rates and stainless tube lengths were used to set three times of heat exposure. Sample 2 was heated to 96° C for 8 seconds, immediately cooled to 4° C, bottled and stored at -20° C. Sample

3 was heated to 96° C for 120 seconds, cooled to 4° C, bottled and frozen. Sample 4 of the late season juice was pasteurized for 180 seconds at 96° C. It was not cooled immediately but placed in a stainless steel 5 gallon bucket at room temperature, covered, and allowed to gradually cool for 2.5 hours. This juice was 68° C when bottled as before and frozen to -20 ° C. Sample 4 of the early-mid juice was initially heated to 96° C for only 60 seconds, bottled and frozen immediately. This juice was thawed on 22 April 1997, heat treated at 96° C for 180 seconds. It was then stored in a covered 5 gallon stainless steel bucket at ambient temperature for 2 hours and was 75° C when bottled and frozen.

Electronic Nose Analysis

Examination was conducted with a Neotronics eNOSE 4000 equipped with 12 organic polymer sensors (Table 3-1). Frozen juice was quickly thawed by placing the bottle under a stream of 30 ° C water. Twenty mL of juice was placed in glass vessels supplied with the instrument. Samples were maintained at 30° C. The sampling program consisted of a one minute period for temperature stabilization, 2.5 minute sensor head purge with nitrogen (2.5 minute sample equilibration), and 1.5 minute headspace sampling. Four different samples of each juice were replicated three times which resulted in 12 total assays per sample. Further examination of late season unpasteurized and extremely pasteurized was conducted. Frozen juice was thawed and examined in the manner previously described.

Table 3-1 Early-Mid juices: Standardized coefficients for Canonical Variables with Eigenvalues and Cumulative Proportion of Variance Explained by Functions

Sensor	Root 1	Root 2
301	-0.1411	5.0586
298	8.0431	0.0207
297	-6.2816	-1.2432
283	-16.4364	2.9472
278	-1.5507	0.8691
264	11.9037	9.6218
263	6.5546	-13.0773
260	-2.9213	-0.7524
262	-3.7804	1.8209
259	-9.3437	-3.7614
258	15.0345	-1.2439
Eigenvalue	25.7042	1.4374
Cumulative Proportion	92.89%	98.08%

Statistical Analysis

Sensor response at 1.00 minute was imported into Statistica (StatSoft, Tulsa, OK; Version 5). Canonical discriminant analysis was utilized to determine if samples could be grouped in accordance with specified variables such as heat treatment category. When samples were classified as two groups, Mahalanobis distances were employed to determine the distances between the grouping variable center (mean) and cultivar responses. This mean value, or group centroid, was obtained through transformation of data by the linear discriminant function (there is only one discriminant function, or root, when dealing with 2 groups). Data are then averaged to yield a mean. This method allowed for prediction of group membership based on distance to group centroids (Massart et al., 1988). Shorter distance indicated a stronger correlation.

Results and Discussion

Early-mid season

We expected the transformed data from juices exposed to heat for longer times would form groups that separated from juices with less thermal treatment. This was true in most cases, but there were exceptions. Canonical discriminant analysis conducted on sensor responses to early-mid juices (grouping variable = 4 types of heat treatment) indicated 4 separate categories (Figure 3-1). Unpasteurized (EM0) was grouped differently than excessively pasteurized (EM180) or pasteurized for 8 seconds (EM8). There was discrimination between EM0 and pasteurized for 120 seconds (EM120), but it was not as conclusive. It seemed logical EM8 would form a group similar to EM0, and EM120 would more closely resemble EM180. This was not the case and at this time we are uncertain why. There may have been formation of thermally generated volatiles in juices subjected to excessive heat. There may have also been loss of other volatiles in these juices. Table 3-1 indicated sensors most discriminating (those with largest absolute values for standardized coefficients) were 258, 259, 264, 283, and 298. The first discriminant function (root 1) accounted for 92.89% of variance while the second accounted for an additional 5.19%.

Late Season

Canonical discriminant analysis of late season juice sensor data (grouping variable = 4 heat treatments) showed late season juice pasteurized for eight seconds (V8) and late season juice pasteurized for 120 seconds (V120) formed separate groups, but

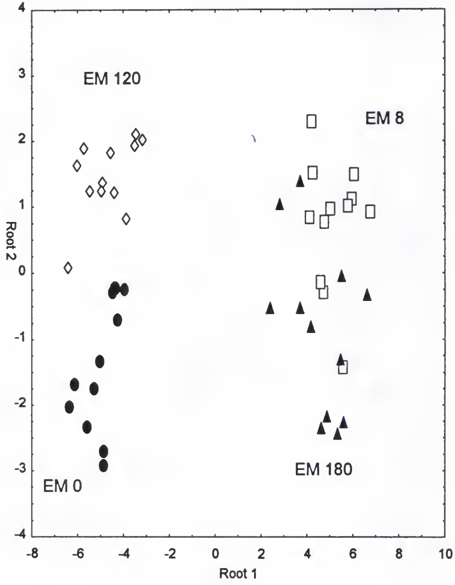


Figure 3-1 Discriminant analysis of early-mid unpasteurized (●), pasteurized for 8 seconds (□), pasteurized for 120 seconds (◇) and pasteurized in excess of 180 seconds (▲)

unpasteurized juice (V0) and pasteurized in excess of 180 seconds juice (V180) appeared to form a single group (Figure 3-2). Table 3-2 indicated sensors most discriminating were 259, 262, 264, 283, and 297. The first discriminant function (root 1) accounted for 97.74% of variance while the second accounted for an additional 1.93%.

Samples of V0 and V180 were re-analyzed to determine if experimental error was responsible for this apparent lack of group separation. When new data were analyzed with Canonical discriminant analysis, results matched the previous findings. There was no discrimination between V0 and V180 groups. However, discriminant analysis with Mahalanobis distances revealed samples were correctly classified with 87.5% accuracy (Table 3-3). Slightly more V0 was correctly classified (91.6%) than V180 (83.3%).

Late Season vs. Early-Mid

Successful discrimination between early-mid season cultivars and late season late season was generally indicated by results of discriminant analysis (grouping variable = 2 juice types, early-mid and late season) with Mahalanobis distances. Juices were properly grouped with an overall accuracy of 81.3%. The classification accuracy of late season juice was 85.4% and for early-mid juice 77.1 %.

Correlation with Other Studies

Fellers and Carter (1993b) reported the amount of heating required to produce off-flavors in orange juice is much greater than required to simply reduce microbial and enzymatic activities. Typically eight seconds at 96° C is sufficient pasteurization for

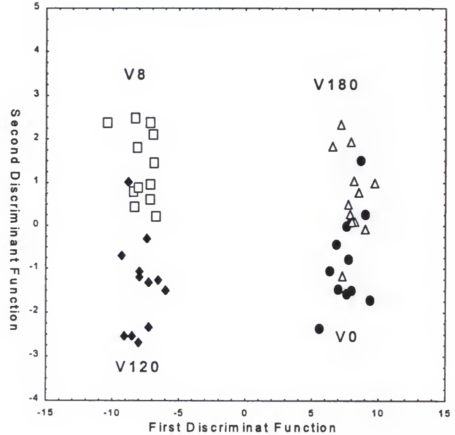


Figure 3-2 Discriminant analysis of late season unpasteurized (●), pasteurized for 8 seconds (□), 120 seconds (◇), excess of 180 seconds (△)

microbial and enzymatic deactivation (King, 1997). Immediate off flavors were noted only after heating to 100° C for 10 minutes. Their study concluded some juice in the U.S. is very overprocessed, because it contains thermally generated off-flavors.

Table 3-2 Late Season Juices: Standardized Coefficients for Canonical Variables with Eigenvalues and Cumulative Proportion of Variance Explained by Functions

Sensor	Root 1	Root 2
301	-1.7152	5.1979
298	-0.9057	-7.0038
297	4.0919	3.3545
283	-10.3245	12.8567
278	-2.3993	-3.141
264	13.6772	7.257
263	-2.9207	-20.3982
260	-0.7362	-1.40158
262	-5.3693	4.6209
259	5.4231	-2.2044
258	1.1546	0.9043
Eigenvalue	67.0099	1.3247
Cumulative Proportion	97.74%	99.67%

Table 3-3 Mahalanobis Distances of V0 and V180 from Group Centroid

Sample	Distance from group V0 centroid	Distance from group V180 centroid	Classified	Predicted
V0	12.33	14.55	1	1
	4.3	10.33	1	1
	13.03	27.37	1	1
	17.19	29.11	1	1
	7.42	17.19	1	1
	12.97	25.65	1	1
	12.38	13.82	1	1
	7.19	9.76	1	1
	6.48	11.6	1	1
	9.27	16.76	1	1
	10.08	5.8	1	2
	6.96	6.37	1	2
V180	18.81	11.35	2	2
	19.3	10.74	2	2
	22.12	14.1	2	2
	17.41	11.5	2	2
	14.56	6.79	2	2
	18.85	11.64	2	2
	15.56	10.22	2	2
	11.12	8.34	2	2
	7.87	11.5	2	1
	16.96	14.36	2	2
	15.1	6.46	2	2
	13.34	5.29	2	2

In our study, we wanted to ensure the thermal generated off-flavors were present in abundance in the sample exposed to the most heat (3 minutes at 96° C, then slow cooled in a stainless steel container for 2 hours). Based on flavor, it is likely some juice processed commercially undergoes similar treatment. Hodgins and Conover (1995) reported conducting polymers are highly sensitive to many of the compounds that evoke an aroma response in the human nose, but these polymers may respond to volatiles that are not detected by the human nose. Our results indicated sensors responded in a similar manner to volatiles of late season unpasteurized and severely pasteurized juices. Initial sensory screening of these 2 samples indicated there was a large difference in aroma. Volatile composition responsible for aroma obviously changes upon severe heat treatment. Levels of alpha-terpineol and furfural increase whereas levels of linalool, neral, geranial, octanal, and decanal decrease (Lee and Nagy, 1996; Durr et al., 1981). Depending on sensor specificity, signals from components with similar chemical structure, although possessing strikingly different aromas, may induce like responses.

Implications

Poor orange juice quality due to thermally generated flavor defects has long been a serious problem for juice processors. Current quality control screening consists of measuring the percent acid and soluble solids of juice following extraction and prior to pasteurization or concentrating. We have shown that statistical analysis of organic sensor data can discriminate between orange juices heated for different lengths of time. However, the separation between groups does not always follow a pattern based solely on time of heat treatment. Greater sensor specificity to volatiles in fresh and heat abused orange flavor is needed before such a tool could provide a reliable system to distinguish between a high quality and low quality juice. Once developed, this technology would be a major asset to the citrus processing industry.

Future Work

Studies focusing on quantification of specific aroma active volatiles which are most important in fresh fruit juice flavor and heat abused flavor are needed. Once a desirable aroma profile is acquired, development of sensors specific to important aroma volatiles will be needed to make electronic nose technology a more useful tool for juice processors.

CHAPTER 4

COMPARISON OF UNPASTEURIZED ORANGE JUICE ANALYSIS BY "ELECTRONIC NOSE," SENSORY TASTE PANEL AND HEADSPACE GC

Introduction

Orange juice aroma and taste quality are important to consumer acceptance. Historically, evaluation of orange juice quality has been conducted by sensory panel testing and/or by capillary gas chromatography with flame ionization detection (Civille and Lyon, 1996; Nawar, 1966; Paik and Venables, 1991a; Lin et al., 1993). Sensory methods employed in taste panels include simple hedonics, difference from control, and descriptive analysis (Civille and Lyon, 1996). Gas chromatography has been the method of choice to analyze citrus juice volatiles. Typically, extraction of volatiles is conducted with either liquid-liquid, dynamic, or static headspace extraction (Paik and Venables, 1991a; Moshonas and Shaw, 1986).

The effect of storage time on the rate of change in orange juice flavor will depend on storage and/or processing conditions. Reconstituted juice flavor quality deteriorates at a steady rate (Dougherty et al., 1974; Fellers and Carter, 1993), whereas pasteurized juice exhibits less linear change in quality (Fellers and Carter, 1993). Unpasteurized juice deteriorates more rapidly than pasteurized or reconstituted juices due to spoilage microorganisms and degradative enzymes. Despite the relatively limited shelf life, the initial superior flavor of the unpasteurized product has resulted in a growing commercial market. By quantifying volatile components from GC data and applying pattern recognition techniques, unpasteurized juice may be discerned from pasteurized and reconstituted juices (Shaw et al., 1993)

Since its initial development and subsequent commercial production, "electronic nose" technology has become increasingly sophisticated and should be able to discriminate between samples differing in volatile composition. "Electronic nose" instruments were utilized to monitor blueberry quality (Simon et al., 1996), classify commercial coffees and tainted water samples (Singh et al., 1996), distinguish between coffee blends (Gardner, 1992) , and to identify paper quality (Holmberg et al., 1995).

Our objective was to compare the ability of a sensory panel, an "electronic nose" and capillary GC static headspace to discriminate changes in unpasteurized orange juice flavor during storage at 2° C.

Materials and Methods

Commercially extracted unpasteurized orange juice was purchased on 15 July 1996 in Gainesville, FL. The juice was eight days old at the time of purchase. Originally packaged in half gallon plastic jugs, all juice was combined in stainless steel 5 gallon buckets and thoroughly mixed to produce a homogenous product. Following blending, new half gallon polyethylene containers were filled with juice (no more than 2 inches headspace), sealed, and stored at 2° C. Day 0 samples were immediately frozen to -29 °C. Day 3 samples remained at 2° C for 72 hours before being frozen at -29° C. Similarly day

6 samples were frozen after 144 hours, day 9 samples after 216 hours, and day 12 samples after 288 hours storage.

Electronic Nose Analysis.

Juice samples were removed from frozen storage and placed in a cooler maintained at 13° C 24 hours prior to analysis to thaw the product. Samples were mixed before opening. Initial "electronic nose" analysis was conducted on 2 August 1996 with an AromaScan instrument (model A32S: AromaScan; Hollis, NH). Analyses were conducted in triplicate. Twelve days later, different lots of the same juice were freshly thawed and electronic nose data was replicated. Sensory panel analysis was also conducted on this day.

For each of three replicates, 200 mL of juice was placed in a sampling jar (500 mL Duran bottle with a three way valve Omnifit cap). Atmospheric air was supplied into the sample to create a dynamic headspace. This was facilitated by connecting the air pump on the AromaScan instrument to the headspace sampling jar with a small (12cm x 3mm i.d.) piece of plastic tubing. Air was bubbled through the juice at 150 mL / min.

Sample analysis followed a set routine called "automatic valve sequence" which was as follows: reference air was sampled for 30 seconds, orange juice sampled for 120 seconds, sensors were washed with an alcohol solution for 30 seconds, reference air was again sampled for 180 seconds. Following completion of each analysis run, detector output was saved in the computer data files for subsequent calculations, graphing, and comparison.

Wilks - Lambda, the standard statistic used to denote statistical significance of the discriminatory power of a model (StatSoft, Tulsa, OK; version 5) indicated sensors 1, 5, 17, 19, 21, 22, 23, 24, 26, and 32 most significantly contributed to separations between samples. Canonical discriminant analysis was conducted on data from these sensors.

Sensor response was normalized by the following:

$$\left[\frac{\text{average sensor response - response to reference air}}{\text{sum of numerator values for all 32 sensors}}\right] \text{X } 100$$

Canonical discriminant analysis employed normalized data.

SPME Extraction and GC FID Analysis.

Extraction of orange juice volatiles was conducted by SPME with a 200um poly(dimethylsiloxane) fiber (Supelco, Bellefonte PA). Ten mL of orange juice was placed in a 40 mL glass vial with a plastic screw cap and Teflon coated septum. A magnetic stirring bar was placed in the vial and the sample stirred vigorously. Samples were maintained at 28 °C for 30 minutes to reach headspace equilibrium. After insertion into the vial, the coated fiber was exposed to the orange juice headspace for a 40 minute equilibration period (Steffen and Pawliszyn, 1996b). The SPME fiber was then removed from the sample's headspace and injected through the GC injection port which contained a narrow bore (1mm) liner. The GC (HP 5890) was equipped with a 30m x 0.25mm i.d. DB5 capillary column (J&W Scientific, Folsom, CA). Operating conditions were as follows: column temperature was held at 32 ° C for 3 minutes and then increased at a rate of 6 ° C /minute to 200 ° C. This temperature was maintained for 1 minute before returning to initial temperature. Injection and detection port temperatures were 250° C. Helium carrier gas linear velocity was 29 cm/sec. Canonical discriminant analysis was conducted on 16 selected FID peak areas to determine if samples could be grouped based on volatile profile and storage time.

Sensory Analysis.

Sensory analysis was conducted utilizing a difference from control format. Panelists were presented with six samples. One was labeled "control" which was the day 0 juice. The other samples (day 0, 3, 6, 9, and 12) were coded with random three digit numbers and randomly arranged on serving trays when presented. Thirty-one untrained panelists were asked to rate differences between the control and each of the other samples on a 0 - 15 point scale with 0 indicating no difference and 15 indicating extreme difference. An analysis of variance (SAS for Windows, VER 6.11; Cary, NC) was conducted on panelist responses.

Results and Discussion

Multisensor Volatile Analysis.

Canonical discriminate analysis of initial "electronic nose" sensor response indicated that samples were grouped according to storage times (Figure 4-1). A replicate set of juices analyzed 12 days later indicated the presence of sensor drift. Other researchers working with food volatiles have reported changes in sensor response with time (Mielle, 1996) . We had no means of calibrating this instrument to compensate for sensor drift.

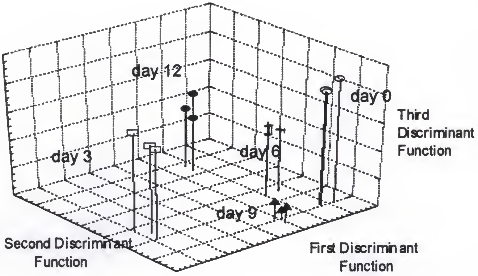
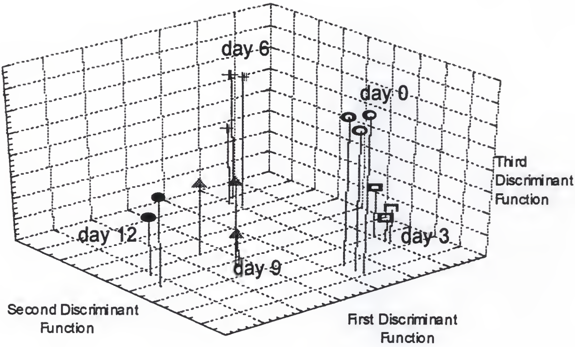


Figure 4-1 Cannonical discriminant analysis of electronic nose sensor data from juices aged for 0, 3, 6, 9, and 12 days at at 2° C.

GC FID Headspace Analysis

Three replications of static headspace analysis were conducted for each juice (with the exception of the sample stored for 12 days which was replicated twice) and relative standard deviations of peak areas ranged from 0.4% to 59% with an average of 27%. GC FID peak areas found in each of the 5 samples were utilized as a "volatile fingerprint" in a manner similar to the electronic nose sensors. No attempt was made to identify FID peaks. Canonical discriminant analysis of selected FID peak areas clearly showed differences between all samples. Figure 4-2 indicted a pattern in which sample position generally changed from upper right to lower left based on increased length of time stored.



Sensory Analysis

An analysis of variance on sensory results indicated there was a linear increase in the difference from the control during storage (Figure 4-3). This mirrored GC findings. There were significant differences between responses for the day 0 juice and all others, $(p<.05)$.

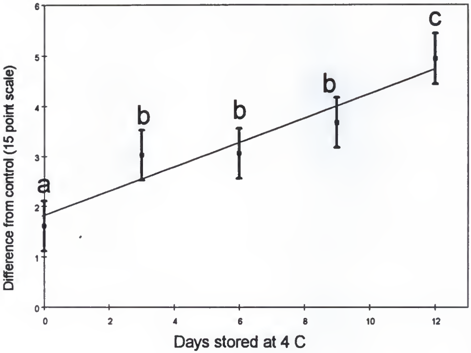


Figure 4-3 Analysis of variance on sensory panel results indicated there were significant differences (p<.05) between the juice frozen at day 0 (a) and all others (b, c), and between the juice frozen at days 12 (c) and all others (a, b).

There were no significant differences between responses for samples stored for 3, 6, or 9 days. The sample stored for 12 days significantly differed from others. Some panelists reported a slight fermented flavor in this sample.

Method Comparison

A comparison of "electronic nose", sensory panel and GC results indicated similarities. Canonical discriminant analysis of both GC and electronic nose data showed distinct separation between samples. However, unlike the pattern exhibited from GC analysis of samples (Figure 4-2), samples analyzed with the electronic nose were not grouped in a general pattern ranging from one quadrant to another based on time of storage (Figure 4-1). Sensory results indicated a general pattern similar to that of GC analysis where juices could be placed in groups classified according to the storage time; however, sensory was less successful and could classify juices in three groups: early, mid and late.

Temperature and Humidity Control

The electronic nose used in this study controlled sample temperature by means of a heated chamber located in the device's interior. However, headspace gases could not be analyzed until the sample was removed from the chamber. Because samples could be heated more quickly in a constant temperature water bath with the same convenience, the heated chamber was not used. All 32 sensors responded to water vapor therefore, the control of humidity was of critical importance.

Advantages of the polymer sensors include greater sensitivity than other sensors such as metal oxide (Gardner and Bartlett, 1994), but this includes greater susceptibility to interfering effects of humidity. This is a potential problem when analyzing samples consisting largely of water, such as fruit juices.

Conclusion

The 32 polypyrrole sensor array detector allowed for the discrimination of unpasteurized orange juice samples stored for different periods of time similar to the results from an untrained sensory panel and headspace GC. Due to the lack of a standardizing procedure to compensate for sensor drift, long term stability is a concern. Once a standard method to compensate for sensor drift has been developed, this technology should enjoy great success in quality control applications.

CHAPTER 5

ANALYSIS OF EARLY-MID SEASON ORANGE JUICE VOLATILES USING SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-OLFACTOMETRY

Introduction

Most gas chromatography-olfactometry (GCO) experiments are conducted using concentrated samples obtained from liquid-liquid extraction. A method of extracting food volatiles that would not mask important early eluting components within a solvent peak would provide the ability to evaluate these potentially important compounds. Solid phase microextraction (SPME) is a rapid, solventless method that provides this ability. SPME contains a fused silica fiber with an outer coating of polymeric organic liquid or solid (Zhang and Pawliszyn, 1993). In headspace analysis the coated fiber is inserted into the vapor phase (headspace) above the sample. Volitalized components adsorb to the polymeric material and are extracted from the headspace. The fiber is then removed and the absorbed volatiles thermally desorbed in a heated injection port of a gas chromatograph for analysis. Compared to dynamic purge and trap, liquid-liquid, simultaneous distillation/extraction, and traditional static headspace extraction, SPME is low cost, rapid, and portable. Carboxen-PDMS fibers are relatively new and designed for extraction of highly volatile components not normally extracted with PDMS alone.

Golascewski (1998) identified 15 headspace components in three varieties of strawberry using SPME equipped with a PDMS silica fiber. Field et al. (1996) successfully extracted humulene and caryophyllene from the headspace of female hop cones and male hop lupulin samples using SPME. Additional studies conducted with SPME include extraction of pyrazines in model systems (Ibanez and Bernhard, 1996) and measurement of caffeine levels in beverages (Hawthorne et al., 1994). Mingyu Jia et al. (1998) evaluated orange juice headspace volatiles using a PDMS coated fiber. They reported optimum equilibration time decreased with increasing temperature, but the quantity of components adsorbed decreased with increasing temperature. Improved chromatographic resolution was observed when the injection port liner diameter was reduced from 1 to 0.75 mm. Steffen and Pawlisizyn (1996) examined 17 volatile components from orange juice using SPME extraction with two different fiber coatings (PDMS and polyacrylate). Polyacrylate was more successful in extracting highly polar compounds such as methanol and ethanol, whereas PDMS extracted both polar and non-polar compounds including terpenes. Yang and Peppard (1994) tested a PDMS coated fiber with twenty-five flavor compounds including ethyl butanoate, linalool, limonene and vanillin. PDMS was more selective for certain compounds than others. It was estimated linalool could be detected at levels of 0.1 - 10 ng/mL, whereas vanillin was not detected at 1 μg/mL. The same authors compared SPME with liquid-liquid extraction (dichloromethane, DCM) of a fruit beverage, and found SPME values equal to or higher than DCM extraction values for some esters, and for many terpenes and terpene alcohols.

Successive Dilution Gas Chromatography-Olfactometry (GCO)

Gas chromatography-olfactometry techniques determine which volatiles have aroma activity, and provides aroma descriptors for each component. Relative aroma intensities are determined from successive dilutions of the extract. Marin et al.(1992a) used GCO to evaluate changes in orange juice volatiles after contact with plastic packing polymers. They reported linalool, citral, vanillin, two ethyl esters, and ten unknown components were major orange juice aroma impact components.

More recently, Tonder et al. (1998) investigated volatiles present in two juices reconstituted from Brazilian concentrate and stored under different conditions. In another recent study, Hinterholzer and Schieberle (1998) identified the most intense aroma active components in hand squeezed, unpasteurized Valencia juice using aroma extract dilution analysis (AEDA). The relative aroma strength of forty-two components were reported.

Time Intensity GCO

Osme is a time intensity GCO method that measures aroma strength and character and does not require dilutions. Previous studies conducted with Osme included aqueous orange essence (Bazemore, 1995), and Pinot Noir wine (Miranda-Lopez et al., 1992).

This study was conducted to determine if SPME using a carboxen-PDMS fiber could successfully extract headspace volatiles for GCO analysis of unpasteurized and heat abused early-mid season orange juices.

Materials and Methods

Mature early-mid season oranges (Hamlin, Pineapple, and Parson Brown cultivars) were harvested from four central Florida groves on 15 and 16 January, 1997. Following harvesting, fruit was immediately shipped to Sun Pure corporation (Sun Pure; Avon Park, FL) where AMC extractors (models 700 and 505; Covina, CA) were utilized for juicing. The juice used in this study contained 11.3 °brix and 0.61% acid.

Heat Treatment

Unpasteurized juice was immediately stored in 1L amber glass bottles with Teflon screw caps and frozen at -20 ° C. Heat treatment of the second sample was conducted with a Microthermic UHT / HTST pasteurizing system, model 35 (Microthermic Corp, Raleigh, NC). Flow rate and stainless tube lengths were used to set the time of heat exposure to 96° C for 60 seconds. Juices were bottled and frozen immediately. A portion of the juice was thawed on 22 April, 1997 and heat treated at 96° C for an additional 180 seconds. It was then stored in a covered 5 gallon stainless steel bucket at ambient temperature for 2 hours and was 75° C when bottled and frozen.

Microbiological Count

Pour plates were prepared with orange serum agar (Redd et al., 1977). Samples were incubated at 30° C for 48 hours to determine if any microbiological activity was occurring in frozen juices.

SPME Extraction and GC FID / MS Analysis.

Extraction of orange juice volatiles was conducted by solid phase micro extraction equipped with a 75µm carboxen-PDMS fiber (Supelco, Bellefonte, PA). Twenty-five mL of juice was placed in 40mL glass vials with plastic screw caps and Teflon coated septa, warmed to 40° C, and gently mixed with a stir bar. Samples equilibrated for 5 minutes prior to insertion of fiber and were maintained at 40° C throughout the 35 minute assay. The coated fiber was exposed to orange juice headspace for a 30 minute equilibration period (Steffen and Pawliszyn, 1996a). The fiber was then removed from the sample's headspace and injected into the GC. An HP 5890 GC equipped with a 30m x 0.25mm I.D. DB5 capillary column (J&W Scientific; Folsom, CA) was utilized. Operating conditions were as follows: column temperature was held at 32 ° C for 3 minutes and then increased at a rate of 6 ° C /minute to 200 ° C. Helium carrier gas linear velocity was 29 cm/sec. A 0.75mm injector liner was utilized for FID analysis. Analysis was conducted in splitless mode.

Component identification was with a Finnigan GCQ ion trap mass spectrometer (Finnigan, Palo Alto, CA) equipped with a 60 m 0.32 mm I.D. DB 5 capillary column (J&W Scientific, Folsom, CA). Identification was confirmed by matching retention indices and aroma characteristics with chemical standards.

Liquid-Liquid Extraction

Juices identical to those extracted with SPME were extracted with pentane : diethyl ether in the manner described by Parliment, (1986), and modified by Klim and Nagy (1992) and Jella et al.(1998). Sixteen mL of juice was added to 5mL of n-pentane and 5mL of diethyl ether and mixed between two syringes connected with stainless steel tubing. The resulting emulsion was centrifuged for 5 minutes (7500g), the upper solvent layer was concentrated to 100 µL and 0.4 µL injected into the GC. For an internal standard, ten ppm propyl benzene was added prior to juice extraction. GC analysis was conducted using the identical temperature program.

Osme Analysis

Panelists selected for participation included 2 males and 1 female. Training consisted of practice runs with a standard solution of eleven components typically found in orange juice (ethyl butanoate, cis-3-hexenol, trans-2-hexenal, alpha pinene, myrcene, linalool, beta-citronellol, carvone, terpin-4-ol, geranial, and neral). Analysis of standards served to familiarize panelists with the Osme aroma intensity scale, optimum positioning and breathing technique, and to provide practice with verbal descriptors. For additional experience, commercial orange juices were evaluated approximately 10 times under identical conditions later used in data collection. Consistency in performance was stressed during training sessions. Test data were recorded only after panelists demonstrated an ability to replicate analyses in a reliable manner.

Osmegrams were developed by first identifying which aroma active peaks were detected by each panelists 2 out of 3 replications. Peaks that met this criteria were then compared with peaks detected by other panelists. Only aroma peaks that were detected by 2 out of 3 panelists were included in consensus Osmegrams. Identification of aroma active components was made by a comparison of retention indices for aroma peaks (Osme analysis) with peak indices from FID and MS.

GC-Olfactometer

Olfactometry was facilitated through a plumbing modification of the Hewlett Packard 5890 gas chromatograph. The capillary column outlet was connected to a line of humidified air. The column effluent-humidified air mixture flowed through a stainless tube (70 cm length, 1 cm I.D.). Humidified air was obtained by directing compressed air through activated charcoal then sequentially through drierite and molecular sieve 5A (Alltech, Deerfield, IL). Purified air was then directed through a temperature controlled, water filled round bottomed flask fitted with input and output glass fittings. Humidified, purified air was adjusted to a flow rate of 11 L / min. Panelists were seated in front of the GC at a position which permitted maximum comfort. Components were separated in the capillary column and carried through stainless steel tubing to the seated panelist who rated the intensity of the volatiles on a 16 point sliding scale (variable resistor). The aroma intensity scale ranged from no aroma perceived = 0, to moderate = 7, to extreme = 15. The sliding scale was interfaced with a personal computer thus allowing time intensity data recordings. At the time of aroma perception, panelists verbally described the aroma to the experimenter. Retention times and verbal descriptions were recorded to permit aroma descriptors to be coupled with computerized aroma time intensity plots. N-alkane standards with retention times encompassing and bracketing retention times of the sample components were analyzed by GC FID prior to olfactometry analysis. This allowed for the conversion of aroma active peak retention times (recorded in minutes) to standard Kovats index values. These standard values could then be related to indices obtained from

MS sample analysis and compared with literature values from compounds of known identity.

Results and Discussion

Osme Analysis

Osme data indicated there were a total of 29 separate aroma active peaks: 24 in unpasteurized juice, and 23 in juice pasteurized in excess of 180 seconds (Table 5-1). Average aroma intensity from consensus Osmegrams for the unpasteurized sample was slightly greater than the pasteurized sample (6.8 and 6.3 respectively using a 15 point scale). Eighteen peaks were common to both samples. Six aroma peaks were lost due to pasteurization and five new peaks were formed during pasteurization. The ten strongest aroma active peaks common to both samples listed in decreasing strength were: ethyl butanoate, myrcene, (E)-2-nonenal, decanal, octanal, two unknowns, terpin-4-ol, (Z)-hex-3-enal, and an unknown (Table 5-2). The unique peaks for the unpasteurized sample (peaks 1, 3, 9, 19, 25, 27) possessed buttery, fruity-floral, terpene, musty, cilantro, and moldy aromas. For the pasteurized sample unique peaks apparently formed during heat treatment (peaks 2, 8, 17, 20, 28) were described as vinyl, rotten fruit, cooked grain, piney and dead bug.

Thermally generated off flavors in orange juice have commonly been attributed to such components as α-terpineol, 4-vinyl guiaicol, and Furaneol (Tatum et al., 1975). Tonder et al.(1998) conducted GCO analysis of a juice reconstituted from concentrate into 1 L TetraBrik containers. One portion was stored for 2 weeks while another portion was stored between 9-12 months. Although the juice stored for 9-12 months had more α-terpineol (1.15mg/L vs. 0.33 mg/L), there was little difference between juices in α-terpineol GCO aroma values. GCO analysis was conducted with a DB5 capillary column where α-terpineol and terpin-4-ol have close (1189 and 1177 respectively) Kovats retention indicies (Adams, 1995). Buttery (1978) lists the aroma thresholds of terpin-4-ol and α-terpineol in air as nearly equal (350 ppb and 340 ppb respectively). Both components were identified in fresh and pasteurized juices, but peak #8 in Table 2 was identified as terpin-4-ol after both components were evaluated by injecting a standard of each into the GC. The juice aroma was much more similar to that of terpin-4-ol than α-terpineol. The aroma of terpin-4-ol was judged stronger in the heat abused juice than in the fresh juice (8.9 vs. 7). Furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone), reported by Hinterholzer and Schieberle (1998) as present in hand squeezed juice, and reported by Tatum et al. (1975) as present in heat abused juice, was not detected in either sample in this study. This may be attributed to the polar character and low vapor pressure of Furaneol and the decrease in polar molecule extraction potential exhibited by the SPME fiber. 4-vinyl guiaicol, not mentioned by Tonder et al. (1998), was detected by our panelists in the heat abused juice, but not in fresh juice (peak #28, Table 5- 2). Aroma activities of selected components in unpasteurized juice were indicated in a plot comparing a FID chromatogram with an Osmegram (Figure 5-1).

Microbiological Counts

Following incubation at 30° C for 48 hours, approximately 4000 colonies were detected in unpasteurized and less than 10 colonies in pasteurized orange juice. Both values were less than the amount required for significant flavor changes in orange juice (Murdock and Hatcher, 1975). Considering juices used for analysis were maintained at -20 ° C until thawed and extracted, the quantity of microrganisms in juice were much less than those incubated.

Comparison of Extraction Methods

As reported by Yang and Peppard (1994) and Steffen and Pawliszyn (1996), SPME was more selective for nonpolar components. Terpenes were extracted effectively, but most early eluting, low molecular weight components were not, nor were larger sesquiterpenes. From SPME headspace extraction of unpasteurized juice, limonene, α-pinene, and myrcene (all terpenes) accounted for 85.5% of total GC FID peak area. From pentane: diethyl ether extraction of unpasteurized juice, the same components accounted for 24% of total GC FID peak area. Pentane:diethyl ether extracts contained many more early eluting components and sesquiterpines.

Extraction Times and Injection Port Temperatures

Yang and Peppard (1994) extracted a fruit juice sample (salt added) with PDMS for ten minutes at ambient temperature. Jia et al. (1998) reported that attainment of an equilibrium between orange juice flavor volatiles and SPME (PDMS) coating required 30 minutes at 40° C. With carboxen-PDMS, early eluting peaks such as acetaldehyde were extracted more efficiently at shorter exposure times, but larger components such as terpenes and sesquiterpenes required a longer equilibration period and were extracted more effectively with times greater than 15 minutes (Figure 5-2). Chromatographic reproducibility as measured by GC FID peak areas was dependent on injection port temperature. At 250° C, relative standard deviation ranged from 13% to 46% (average 26%) for ethyl butanoate, myrcene, linalool, and alpha terpineol peaks. At 320° C the range was from 2% to 34% with an average of 13% for the same components. Although % RSD was better at 320° C, early eluting peaks were much smaller (Figure 5-3). Acetaldehyde accounted for 0.05% of the total peak area at 320° C and 0.48% of the total peak area at 250° C. Poor replication may have been, in part, due to incomplete desorption of analyte from the carboxen-PDMS fiber. Shirey, (1998) reported components larger than C12 are retained strongly on the fibers' surface and are not easily desorbed. FID data indicated % RSD for smaller components (ethyl butanoate) were generally smaller than for larger terpenes, terpene alcohols and sesquiterpenes. A decrease in %RSD with increase in injection port temperature appeared to support this observation.

FID chromatograms indicated early eluting components were extracted more effectively with shorter (3-5 minute) fiber exposure times. Later eluting components were extracted more effectively with longer times (15-45 minutes). During GCO analysis, panelists reported more peaks, and peaks of greater intensity, when extractions were conducted at times over 15 minutes. Shorter extraction periods were found to result in smaller numbers of aroma active peaks. For this reason an extraction time of 30 minutes was utilized for all GCO analyses.

Conclusion

Carboxen-PDMS SPME can be used as a convenient tool for GCO analysis. Osme panelists were able to reproducibly detect six aroma peaks lost upon heat treatment, five aroma peaks formed from heat treatment, and 18 aroma peaks common to both orange juice samples. Shorter extraction time (5 minutes) and lower injection port temperature (250° C) resulted in FID chromatograms with larger early eluting peaks from lower molecular weight components such as acetaldehyde. Longer extraction times and higher injection port temperatures resulted in FID chromatograms with larger terpene and sesquiterpene peaks, and better precision.

Table 5-1 Results from GCO analysis of early-mid unpasteurized and excessively pasteurized juices

			DB5 capillary column		Aroma intensity (0-15)			
Peak #	**Component Identity**	**Aroma Descriptor**	**Kovats Index**	**Retention Time (minutes)**	**Unpasteurized**		**Pasteurized**	
					Mean	Std dev.	Mean	Std dev.
1	2-methyl propanoic acid*	Buttery/c.rice	594	2.8	1.4	0.4	0	N/A
2	unknown	Vinyl/cotton candy/engine/	689	4.9	0	N/A	3.3	0.6
3	3-ethoxy-1-propanol*	Fruity/floral	761	7.1	2.1	0.6	0	N/A
4	ethyl butanoate	Fruity/green/ floral	802	8.6	12.1	1.9	8.1	1.6
5	unknown	Fruity/orange peel/floral/ musty	854	10.2	7	0.2	4.1	1.6
6	(Z)-hex-3-enal*	Medicine/ musty/fruity/ grape	870	10.8	5.8	5.5	7.8	3.9
7	propyl butanoate*	c.rice/terpene/ apple sauce/fruity/ fruity/musty	898	11.8	6.1	0.7	3.3	0.2
8	unknown	Rotten fruit/musty/ minty	912	12.3	0	N/A	3.8	2.1
9	unknown	Terpene/pine/ citrus/ mushroom	979	14.4	6.1	3.6	0	N/A

Table 5-1 (continued) Results from GCO analysis of early-mid unpasteurized and excessively pasteurized juices

			DB5 capillary column		Aroma intensity (0-15)			
Peak #	**Component Identity**	**Aroma Descriptor**	**Kovats Index**	**Retention Time (minutes)**	**Unpasteurized**		**Pasteurized**	
					Mean	Std dev.	Mean	Std dev.
11	myrcene	terpene/ metallic/piney/ earthy/unripe mango/citrus	993	14.9	9.5	1.5	11.9	3.6
12	unknown	Metallic/nutty/ musty/fruity/	998	15.1	7.6	0.9	7.4	2.2
13	octanal	lemon/melon/ terpene/ green grassy	1006	15.3	11	2.6	7	1.1
14	(+)-limonene or 1,8-cineole	citrus/linalool/ mintydirt/earth	1039	16.2	6.2	1.5	4.7	3.4
15	methyl octanoate*	mint/citrus/ moldy/musty/	1047	16.5	7.4	2.6	4.1	3.9
16	unknown	Mushroom/ p-menthene-thiol	1074	17.3	7.1	1.4	4	0.3
17	unknown	c.grain/ mushroom	1084	17.6	0	N/A	3.4	0.2
18	linalool	Musty/incense/ cooked/floral	1103	18.2	7.7	3.4	4.1	0.3
19	nonanal	Musty/sulfur note/cooked/	1109	18.4	5.3	4.3	0	N/A
20	unknown	piney/terpene/ stale church/	1154	19.6	0	N/A	4.9	4.1
21	unknown	Stale church/musty/ cotton clothes	1167	20	4.6	1.6	4.7	2.6

Table 5-1 (continued) Results from GCO analysis of early-mid unpasteurized and excessively pasteurized juices

			DB5 capillary column		Aroma intensity (0-15)			
Peak #	**Component Identity**	**Aroma Descriptor**	**Kovats Index**	**Retention Time (minutes)**	**Unpasteurized**		**Pasteurized**	
					Mean	Std dev.	Mean	Std dev.
22	terpin-4-ol	Green/vinyl/ stale church/	1179	20.27	7	0.6	8.9	4.3
23	(E)-2-nonenal*	Musty/terpene/ wet clothes/floral	1199	20.9	6.6	1.4	11.7	4.0
24	decanal	Musty/fruity/ dusty	1208	21.1	5.1	3.6	11.4	0.5
25	unknown	Cilantro/cooked grain/stinky	1213	21.3	7.4	5.6	0	N/A
26	unknown	Musty/cooked stale/dead bug/melon	1223	21.5	10	3.1	9.1	1.7
27	carvone	Moldy/musty/ earthy/floral/ minty	1260	22.4	4.4	1.2	0	N/A
28	4-vinyl guaiacol	dead bug/musty/ rancid oil	1330	24.3	0	N/A	4.4	0.9
29	ethyl decanoate	Roasted meat/rancid oil/cooked apple sauce/paint/ floor cleaner	1391	25.8	5.7	3.1	4.5	2.9

Table 5-2 Ten most aroma active components in Early-Mid orange juice headspace

Peak #	**Aroma identity**	**Descriptor**	**(Kovats) index (DB5)**	**Unpasteurized**	**Pasteurized**
1	ethyl butanoate	fruity	802	12.1	8.1
2	myrcene	metallic	993	9.5	11.9
3	(E)-2-nonenal*	stale	1198	6.6	11.7
4	decanal	musty	1208	5.1	11.4
5	octanal	citrus	1005	10.9	7
6	unknown	metallic	1223	10	9.1
7	unknown	vinyl	983	9.3	8.1
8	terpin-4-ol	stale	1179	7	8.9
9	(Z)-hex-3-enal	medicene	870	5.8	7.8
10	unknown	nutty	998	7.6	7.4

*peak 3 not positively identified

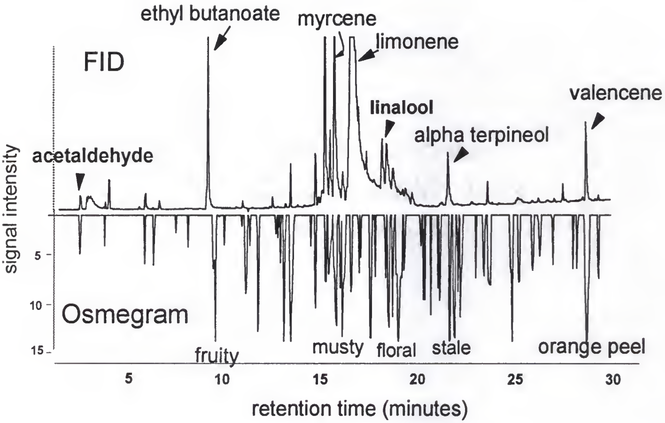


Figure 5-1 Comparison of unpasteurized orange juice FID chromatogram and Osmegram

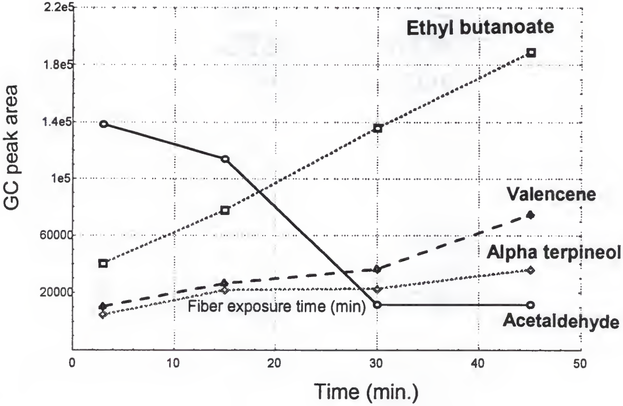


Figure 5-2 Relationship between SPME fiber exposure time and FID peak area for selected components.

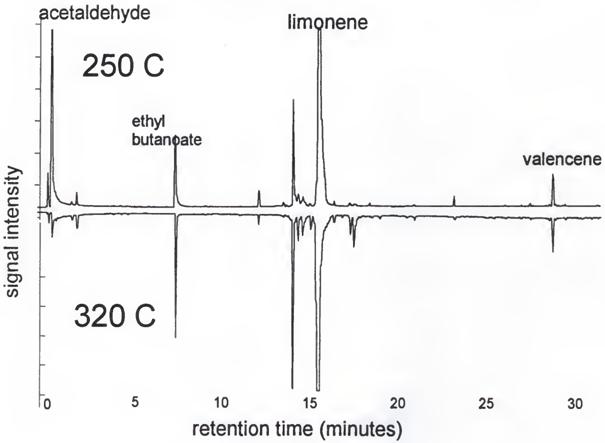


Figure 5-3 Relationship between FID peak size and injection port temperature.

CHAPTER 6

IDENTIFICATION AND CONFIRMATION OF IMPORTANT AROMA ACTIVE VOLATILES IN UNPASTEURIZED AND HEAT ABUSED VALENCIA ORANGE JUICES

Introduction

Orange juice is the most popular juice in the world (Tonder et al., 1998; Shaw, 1991) and the Valencia cultivar provides the most popular orange juice because it typically contains a preferred aroma bouquet and sugar/acid content (Hinterholzer and Schieberle 1998). In the1997-98 growing season, Valencia oranges were worth an estimated $534,192,000 or 61% of Florida orange value, and 55% of the Florida citrus market (Geuder et al., 1998).

Flavor Impact Components

Identification of flavor components in Valencia juice has been the subject of numerous investigations. The majority of studies utilized primarily gas chromatography to investigate volatiles (Rodriguez and Culbertson, 1983; Nisperos-Carriedo and Shaw, 1990; Paik and Venables, 1991b). Some researchers have utilized sensory panels to correlate analytical data from gas chromatography with true human taste perceptions.

Ahmed et al. (1978b) added components back to a solution of orange juice pumpout (near flavorless, viscous material obtained following water removal by an evaporator) in an attempt to produce a beverage with orange juice flavor. They found a combination of acetaldehyde, neral, geranial, ethyl butanoate, *d*-limonene, and octanal added to pumpout in similar amounts found in orange juice produced a beverage of high flavor quality.

Heat Abused Juice Flavor

Heat abused orange juice exhibits an aroma and flavor different from unpasteurized juice. Tatum et al. (1975) employed GC and limited sensory analysis to determine the identity of components important in the flavor of canned, single strength orange juice produced from high temperature storage. They evaluated samples with a 12 member taste panel and concluded α-terpineol, 4-vinyl guaiacol, and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (also known as Furaneol) exhibited malodorous properties and were at least partly responsible for the off flavors associated with stored, canned orange juice. Durr et al. (1981) examined juices stored in glass and plastic with GC and concluded α-terpineol levels increase with increasing storage time and temperature, but limonene, linalool, neral, geranial, octanal and decanal levels decrease. Moshonas and Shaw (1989) investigated the effect of storage time on aseptically packaged orange juice volatiles with GC and with a sensory panel. They concluded α-terpineol concentrations increase with storage time, as did the level of furfural. Ethyl butanoate, octanal, neral, geranial, hexanal, and 1-penten-3-one levels decreased over 8 months of storage. The sensory panel expressed a preference for a control juice stored at -21° C over juices stored at 21° C and 26° C.

Increase in α-terpineol and loss of volatile esters by juices stored at 21° C and 26° C were listed a possible explanation for this sensory preference.

GCO Studies

Studies that investigated orange juice aroma activity with gas chromatography - olfactometry (GCO) were previously reviewed in Chapter 5. None of these studies attempted to add the identified aroma active components back to a model solution (a process called recombination) in order to test the validity of their conclusions. Recombination of key aroma components to test the overall flavor impression has been reported for a few food products. Fischer and Grosch (1981) added the most intense aroma active components identified with Aroma Extraction Dilution Analysis (AEDA) experiments to water and vegetable oil. The legume aroma was best reproduced in vegetable oil and was recognized by ninety percent of panelists as legume-like. Grosch et al. (1994) identified off flavors in butter oil using AEDA. Components were added back to fresh butter oil and the presence of off aroma evaluated, but sensory panel information was not presented.

Descriptive Analysis

Descriptive Analysis (DA) is a sensory method which can be used to measure differences in specific characteristics between samples. Flavor Profiling, a type of DA, was used in this study. In this technique, the panel leader assists panelists in defining flavor terminology to be used in common by the entire panel. Additionally, the panel leader develops and provides reference standards (Meilgaard et al., 1991). Panelists are trained with standards until they can reproducibly rate aroma and taste intensities. Significant differences between samples based on pre-identified sample attributes are evaluated using analysis of variance (ANOVA) coupled with least significant difference (LSD) testing. Advantages of the Flavor Profile method included it's ability to be utilized when many products were evaluated, and as few as five panelists can be used. Lack of consistency and reproducibility was overcome by training and using terms in common to all panel members. Disadvantages of this descriptive analysis method included the possibility that consensus decisions may have been dominated by a single panelist.

The primary objective of this study was to identify important aroma active components in unpasteurized and heat abused Valencia orange juices and to determine their effectiveness in reproducing original juice aroma through recombination.

Materials and Methods

Juice Procurement

Two seasons of mature Valencia oranges were harvested from local central Florida groves. Fruit from the 1997 season was harvested 14 May, 1997, and fruit from the 1998 season was harvested 30 April, 1998. Oranges were shipped to Sun Pure corporation (Sun Pure; Avon Park, FL) where AMC machinery (models 700 and 505; Covina, CA) was utilized for juicing.

Heat Treatment

The heat treated juice was processed with a Microthermic UHT / HTST pasteurizing system, model 35 (Microthermic Corp, Raleigh, NC). Flow rate and stainless tube length was used to set the time of heat exposure to 96° C for 180 seconds. Juice was not cooled immediately but placed in a stainless steel 5 gallon bucket at room temperature, covered, and allowed to gradually cool for 2.5 hours. This juice was 68° C when bottled as before and frozen to -20 ° C.

Volatile Extraction/ Oil Analysis

Juices were extracted by pentane:diethyl ether in the identical method described in Chapter 5. Recoverable oil was measured in the method described by Scott and Veldhuis, (1966)

GCO analysis/Volatile Identification

Osme analysis was conducted in the manner described in Chapter 5. Component identification was made by matching Kovats retention indicies with retention indicies published in literature (Adams, 1995) and with retention indicies of authentic standards, and with mass spectra from a Finnigan GCQ ion trap mass spectrometer (Finnigan, Palo Alto, CA) equipped with a 60 M 0.32 mm I.D. DB 5 capillary column (J&W Scientific, Folsom, CA). The identity of certain volatiles was further confirmed by retention time and Kovats retention index comparisons with a DB-wax capillary column (J&W Scientific, Folsom, CA).

Sensory Analysis

Descriptive Analysis (DA) was conducted with 12 trained panelist (seven males, five females) at the United States Department of Agriculture's Citrus and Subtropical Lab in Winter Haven, FL. Panelists determined which attributes to include on a sensory ballot based on sample characteristics they found most important in distinguishing between fresh and heat abused orange juices. Aroma and flavor intensities were rated on an identical scale as that used in Osme analysis (0-15) with 0 equal none and 15 equal very strong. Attributes listed on the ballot (standards used for familiarization) include floral/fruity (linalool and ethyl butanoate), musty/terpiney (nonanal, terpin-4-ol), cooked (canned commercial orange juice), fresh (unpasteurized orange juice), sweet (sucrose), sour (citric acid). Approximately 20 training sessions focused on recognition and familiarization of standard aromas and their flavors, and their intensities. In addition to standards made from aroma chemicals dissolved in water and ethanol, standards were also made from blending different citrus juices, and from blending different aroma chemicals to orange juices.

Initial Testing

For initial testing, panelists utilized the sensory ballot previously discussed and evaluated three lots of the same Valencia juices: unpasteurized, pasteurized for 8 seconds at 96° C, and heat abused at 96° C in excess of 180 seconds. Samples were labeled with random 3 digit numbers and presented in random order. Examination was replicated and statistical analysis was conducted with analysis of variance (ANOVA) coupled with least significant difference (LSD) testing.

Recombination

For recombination testing, panelists utilized the sensory ballot previously discussed and evaluated an unpasteurized juice previously frozen at -20°C, and two model solutions. The fresh juice model solution contained the eight most aroma intense volatile components identified as important contributors to fresh orange aroma by Osme analysis in a solution of water, citric acid (0.6%w/w), and sucrose (10% w/w). The heat abused model solution contained volatile components important in heat abused aroma, and smaller volumes of components important in fresh aroma, dissolved in the same matrix (Table 5-1). Four additional components, acetaldehyde, ethanol, sinensal, and limonene were later added to both model solutions to improve flavor. Samples were labeled with random 3 digit numbers and presented in random order. This experiment was replicated and statistical analyses were conducted with analysis of variance (ANOVA) coupled with least significant difference (LSD) testing.

Results and Discussion

Brix/Acid and Oil Level

Brix and acid levels for unpasteurized juice were 12.6° and 0.65% respectively. Peel oil level for unpasteurized juice was 0.025%, and for heat abused juice 0.017%.

Table 6-1 Unpasteurized and heat abused model solution components

Component	Model solution 1 (unpasteurized) Ppm	Model solution 2 (heat abused) ppm
Linalool	6	3
Ethyl butanoate	6	3
Octanal	1	1
Valencene	4	2
Myrcene	1	1
Geraniol	4	2
Citronellol	1	1
Acetaldehyde	10	5
Ethanol	10	5
Limonene	20	20
Sinensal	2	0.5
Furaneol*	1	1
Decanal	0	8
Terpin-4-ol	0	8
4-vinyl guiaicol	0	8

Furaneol* = 2,5-dimethyl-4-hydroxy-3(2H)furanone

GCO

A total of 40 aroma active peaks were observed in unpasteurized orange juice. Twenty-six aroma active peaks were found in the heat abused orange juice. The fifteen most aroma active components identified by Osme as important in unpasteurized juice are listed in Table 6-2. The most intense aroma components in heat abused juice are listed in Table 6-3. Two intense Osme aroma peaks observed in both unpasteurized and heat abused juice, one aroma peak found only in unpasteurized juice, and 1 aroma peak found only in heat abused juice were only tentatively identified because standards were not available. These components did match published retention indices and published GC/MS spectra. No new aroma active components were identified in this study. In unpasteurized juice, linalool had the strongest aroma intensity followed by valencene, Furaneol, ethyl decanoate, and an unknown. In heat abused juice an unknown, octanal, linalool, ethyl decanoate, and terpin-4-ol had the strongest aroma intensities. An unpasteurized GC FID chromatogram is paired with a corresponding Osmegram in Figure 6-1.

Comparison of GCO Results with Other Studies

A comparison of our GCO study with two other studies previously conducted with Valencia juice identified several similar findings. Marin et al. (1992b) reported the five most aroma active components in mechanically squeezed, pasteurized Valencia juice to be linalool, citral (the conformational isomers geranial and neral were not differentiated), and 3 unknowns. In hand squeezed, unpasteurized juice, they found 5 unknown components were most aroma active (linalool was absent). Although they did not find linalool in their hand squeezed unpasteurized orange juice, Marin et al. (1992) found linalool to be the most aroma active component in their mechanically squeezed, pasteurized sample. They listed 15 additional aroma active peaks in their study. We confirmed the identity of three of the five they identified. in our samples (vanillin, ethyl butanoate, and citral isomers). Hinterholzer and Schieberle (1998) listed a total of 42 aroma active components in hand squeezed, unpasteurized Valencia juice. The five most aroma intense were: ethyl butanoate, (Z)-3-hexenal, 3a,4,5,7a-tetrahydo-3,6-dimethyl-2(3H)-benzofuranone , ethyl 2-methylpropionate, (S) -ethyl 2-methylbutanoate. We found 12 components in common with the list generated by Hinterholzer and Schieberle (1998). These aroma active volatiles included: ethyl butanoate, linalool, octanal, furaneol, nonanal, decanal, myrcene, (Z)-2-nonenal, (E)-2-nonenal, vanillin, (Z)-octa-1,5-diene-3-one, and methional.

Sensory

Initial juice testing

Panelists were able to distinguish between unpasteurized and heat abused samples in fresh orange flavor, fruity/ floral, musty/ terpene, and cooked processed ($p<.05$). Differences in fresh orange flavor and peel oil/ burn were also indicated between unpasteurized juice and juice pasteurized for 8 seconds at 96° C. Taste attributes (sweet and sour) were not significantly altered by heat treatment. (Table 6-4).

Recombination

Determination of components important in orange juice flavor

Informal sensory screening by three panelists revealed addition of only the most intense aroma active components to single strength pumpout (pumpout: high brix, viscous orange juice product obtained by evaporation of water) produced a model solution whose flavor did not resemble the flavor of orange juice. It lacked the balance and subtleties characteristic of orange. Quantities of aroma components added were based on volumes utilized by Ahmed et al. (1978a) and based on volumes of components in orange juice reported in literature. Identical components and quantities were added to a water, sucrose, and citric acid solution with the same result; a flavor distinctly different from orange juice. Following experimentation with several different components reported in literature as important to orange juice flavor, it was determined acetaldehyde, ethanol, limonene, α-sinensal and βsinensal were required. It was also determined matrix differences between orange juice and a solution of water, sucrose and citric acid affected volatile distribution in headspace. Identical concentrations of volatiles in orange juice and in water, sucrose, citric acid would have different aromas. Of the 5 additional volatile components added, only the sinensals were identified by GCO, but their aroma intensities were not strong (α-sinensal rated 6 out of 15, β-sinensal was less). It was not possible to identify acetaldehyde using GCO coupled with pentane:diethyl ether extraction because the acetaldehyde peak was masked by eluting solvent. Acetaldehyde was responsible for adding a fresh, green aroma, and a burn sensation characteristic of freshly prepared orange juice flavor. Ethanol, the volatile component present in greatest abundance in orange juice (Wade et al., 1992) provides a lift to aroma and flavor (Arctander, 1994). *d*-Limonene, the second most abundant volatile in orange juice, also appeared to be responsible for a lift, and burn sensation. Without limonene the citrus character was severely inhibited. Both sinensals supplied a definitive orange aroma in concentrations as low as 500 ppb.

Fresh model solution (FMS) contained eight of the most intense aroma active components (determined from GCO analysis) added to a solution of sucrose, citric acid, and water. Heat abused model solution (HAMS) contained less of several components added in FMS, but contained decanal, terpin-4-ol, and 4-vinyl guaicol, components not added to FMS because they were identified as more important to the aroma of heat abused juice (Table 6-1).

Table 6-2 Most aroma active components in unpasteurized Valencia juice

Aroma Peak #	Aroma Intensity (0-15) Mean	Aroma Intensity Std dev.	Kovats Indice	Retention Time (minutes)	Aroma Descriptors	Component Identity
1	8.4	2.3	824	8.61	Floral	ethyl butanoate
2	8.3	2.7	951	12.8	c.oats/grain/ diacetyl	alpha thujene*, b-citronellene*
3	7.2	1.3	998	14.4	Mushroom/ earthy/ mango/terpene	myrcene
4	8.7	2.5	1014	14.8	unripe mango	octanal
5	9.8	3.3	1096	17.3	cotton candy	(4-hydroxy-2,5-dimethyl-3(2H)-furanone
6	15	1.6	1112	17.7	Floral	linalool
7	8	0.2	1128	18.1	Mushroom/ roasted. meat /watermelon	nonanal
8	8.4	3.4	1135	18.4	Linalool/ jasmon	unknown
9	7.9	1.5	1150	18.7	Grain terpene/burnt b.scotch	(Z)-2-nonenal*
10	7.3	1.4	1192	20	musty stale/ plastic / wet cotton	decanal and (E)-2-nonenal*
11	8.9	3.0	1270	22	musty/ rancid/ floral	citronellol
12	8.8	2.2	1298	22.7	Orange/ sandelwood	geraniol and carvone
13	9	3.0	1363	24.2	Smokey	4-vinyl guaiacol
14	9.5	2.6	1426	25.7	Rancid oil/ apple sauce/ pepper	ethyl decanoate
15	10.2	3.2	1482	27.7	Orange peel	valencene

Table 6-3 Most aroma active components in heat abused Valencia juice

Aroma Peak #	Aroma Intensity (0-15) Mean	Aroma Intensity Std dev.	Kovats Indice	Retention Time (minutes)	Aroma Descriptors	Component Identity
1	7	2.8	927	12.2	savory/fritoes/ vegies/ rice	2-acetyl-1-pyroline*, methional
2	6.7	4.1	990	14.2	Mushroom	myrcene
3	13	6.4	1001	14.8	Citrus	octanal
4	9.5	6.1	1041	15.7	minty/ fruity	limonene, 1,8-cineole, alpha-phellandrene, ethyl-3-hydroxy hexanoate*, methyl octanoate*
5	7.8	1.5	1084	17	Cotton candy	(4-hydroxy-2,5-dimethyl-3(2H)-furanone
6	12.9	5.6	1108	17.7	Floral	linalool
7	9.2	0.3	1127	18.2	burnt candy	ocimene*
8	6.7	3.7	1157	19	grain/green/ melon/burnt buttercandy	(Z)-2-nonenal*
9	9.7	3.7	1176	19.6	vinyl/ plastic/ musty	terpin-4-ol
10	8.4	4	1204	20.3	piney/ minty/ terpene/ clothes	decanal and (E)-2-nonenal*
11	7.2	0.3	1270	21.9	apple sauce/ chewing gum/ rancid oil/ peanuts/ pasty	carvone, citronellol
12	14.3	6.9	1350	24	Smokey/ charcoal	4-vinyl guaiacol
13	10.2	3.7	1430	26	Pepper/ grape	ethyl decanoate
14	9.1	1.9	1482	27	Orange peel, vanilla	valencene and vanillin

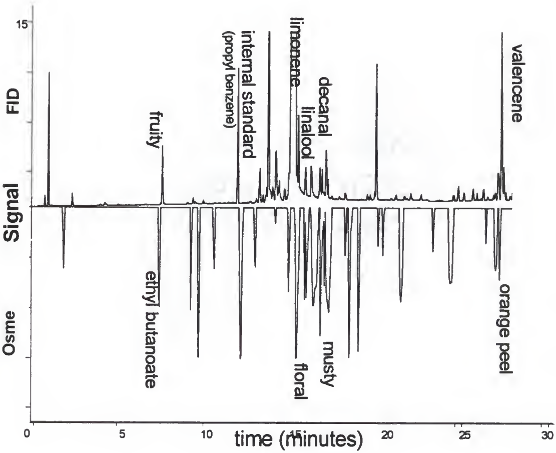


Figure 6-1 GC FID chromatogram (top) and GCO Osmegram (bottom) from unpasteurized Valencia orange juice.

Table 6-4 Mean scores from Descriptive Analysis of three Valencia juices

	Aroma intensity	**Fresh orange flavor**	**Fruity/ floral**	**Peel oil/ burn**	**Musty/ terpene**	**Cooked/ processed**	**Sweet**	**Sour**
Unpasteurized	9.6^{a}	9.8^{a}	8.9^{a}	3.3^{a}	2.8^{a}	2.8^{a}	9.4^{a}	4.6^{a}
Pasteurized for 8 seconds	8.6^{a}	7.2^{b}	$6.8^{a,b}$	5.9^{b}	$3.7^{a,b}$	4.2^{a}	8.7^{a}	5.1^{a}
Heat abused	10.3^{a}	4.3^{c}	4.6^{b}	$4.9^{a,b}$	4.8^{b}	10.2^{b}	8.6^{a}	4.9^{a}

scores with different letter superscripts are significantly different ($p < .05$)

Discriminant analysis of model solutions

DA results of recombined FMS and HAMS, and of an unpasteurized Valencia juice previously frozen are found in Table 6-5. There were no significant differences between samples in fresh orange aroma and flavor, although mean scores were highest for unpasteurized Valencia and FMS. There also were no significant differences between samples for cooked / processed flavor, although HAMS had the highest mean score for the flavor attribute. FMS had a significantly more intense aroma than HAM ($p<.05$). Unpasteurized juice had significantly greater peel oil / burn than FMS probably due to a greater quantity of limonene.

A possible explanation for panelists failing to perceive differences between FMS and HAMS was because the same "fresh" components were used in both solutions. Despite the generally lower concentrations of "fresh components" added to HAMS, they apparently were sufficiently strong to obscure the addition of "heat generated" components. Another possible explanation for panelists failing to distinguish differences in fresh orange aroma, flavor, and fruity floral and cooked/processed aromas in the model solutions was the added "heat abused" components identified by GCO were not important, or only slightly important to heat abused aroma. Not all heat abused aroma peaks were identified, and these components may be required in heat abused aroma. Flavor differences in heat abused and unpasteurized juices may also be due to the presence of less volatile components, or differences in component proportions, or both.

Initially, recombination sensory studies were conducted with pumpout. SPME extraction and subsequent GC analysis of pumpout revealed no volatile peaks, although the pumpout had a strong cooked aroma that obscured subtle, fresh aromas of added components such as linalool, geraniol, and ethyl butanoate.

Conclusion

GCO aids in the identification of aroma active volatile components, but the contribution of some volatiles may not be indicated (acetaldehyde, ethanol, limonene, sinensals). Non-volatile food components (pulp, lipid) may affect the headspace distribution of volatile compounds, therefore they can also be important in the perception of flavor balance.

Table 6-5 Recombination - mean scores

	Aroma intensity	Fresh orange aroma	Fresh orange flavor	Fruity/ Floral	Peel oil/ burn	Musty/ terpene	Cooked/ processed	Sweet	Sour
Model solution 1 (unpasteurized)	11.5^{a}	6.6^{a}	5.5^{a}	7.5^{a}	4.2^{a}	4.9^{a}	3.3^{a}	8.6^{a}	6.1^{a}
Unpasteurized Valencia	$9.8^{a,b}$	6.6^{a}	6^{a}	5.9^{a}	6.6^{b}	4.9^{a}	3.4^{a}	7.6^{a}	6.5^{a}
Model solution 2 (heat abused)	9^{b}	5.4^{a}	4.7^{a}	5.7^{a}	$5.3^{a,b}$	5.9^{a}	3.6^{a}	7.9^{a}	6.9^{a}

scores with different letter superscripts are significantly different ($p < .05$)

CHAPTER 7

SOURCES AND STABILITY OF LINALOOL IN VALENCIA ORANGE JUICE

Introduction

Linalool is a terpene alcohol with a strong floral aroma and is an important contributor to the flavor and aroma of numerous products including perfumes (Arctander, 1996; Bauer et al., 1997), lychee (Ong and Acree, 1998), lemon oil and certain teas (Clark and Chamblee, 1992). It has also been reported as one of the major flavor impact components in mechanically squeezed, pasteurized orange juice, but absent in hand squeezed, unpasteurized orange juice (Marin et al., 1992b).

The reported levels of linalool in fresh and pasteurized orange juice vary from a low of 0.15 ppm to a high of 11 ppm (Maarse and Visscher, 1985). Schreier (1981) used liquid-liquid extraction of orange juice and reported the concentration of linalool to be greater in fresh juice (unpasteurized) than in pasteurized juice. In a broad analytical study, Nisperos-Carriedo and Shaw (1990) analyzed fifteen different juices for headspace concentrations of 20 flavor components, including linalool. "Fresh juice" was a blend of hand-extracted, unpasteurized, Valencia, Hamlin, and Pineapple orange varieties. In addition to fresh juice, they analyzed unpasteurized, commercially prepared juice, pasteurized, not from concentrate juice, pasteurized from concentrate juice, aseptically packaged from concentrate juice stored in glass and cartons, canned juice stored in two types of metal containers, and a juice drink Linalool concentrations ranged from "trace amounts" to 5.3ppm. Concentrations in unpasteurized, fresh juice, and in two of the "highest quality" pasteurized samples were reported as "trace levels". Aseptic packaged, reconstituted from concentrate juices contained the highest quantities of linalool followed by frozen concentrate juices.

In studies reported by Schreier (1981) and Nisperos-Carriedo and Shaw (1990), the effect of juicing method on linalool concentration was unclear. The manner in which juice was extracted from the fruit was not indicated. In the research conducted by Marin et al. (1992), it was unclear if linalool concentrations changed due to method of juicing (hand squeezed vs. mechanically squeezed) or due to heat treatment (unpasteurized vs. pasteurized), therefore, the primary objectives of this study were to determine if linalool originates primarily in orange peel or in juice vesicle cytoplasm, and to determine the effect of heat processing on linalool stability.

Materials and Methods

Valencia oranges were harvested March, 1998 from Citrus Research and Education Center (CREC) groves. Oranges were divided into 3 groups, each contained 60 fruit. To ensure a juice free of peel oil, one group of oranges were peeled, sliced equatorially, then juiced with a mechanical reamer. One-half of this juice was immediately extracted with pentane:ether. In order to measure the effects of heat processing, the other half of juice was pasteurized at 92° C for 30 seconds with a Microthermic UHT / HTST pasteurizing system, model 35 (Microthermic Corp, Raleigh, NC). Another group of oranges (peel intact) were sliced equatorially and juiced with the mechanical reamer. As before, one-half of this juice was immediately extracted with pentane:ether. The other half was pasteurized at 92° C for 30 seconds, then extracted. The final 60 orange group was processed with an FMC single head juice extractor. The juice was divided and handled in the same manner as with the other samples.

Volatile Extraction and GC Analysis / Oil Analysis

Methodology of pentane:diethyl ether extraction and oil analysis were identical to those discussed in Chapter 5.

Sensory

Descriptive Analysis (DA) was conducted in the manner described in Chapter 6. Juices analyzed by DA were peeled-unpasteurized, unpeeled-unpasteurized, and FMC extracted-unpasteurized. Samples were presented in random order and labeled with random 3 digit numbers. Examination was replicated and analysis of variance (ANOVA) coupled with Least Significant Difference (LSD) testing was performed on results.

Results and Discussion

Oil Levels

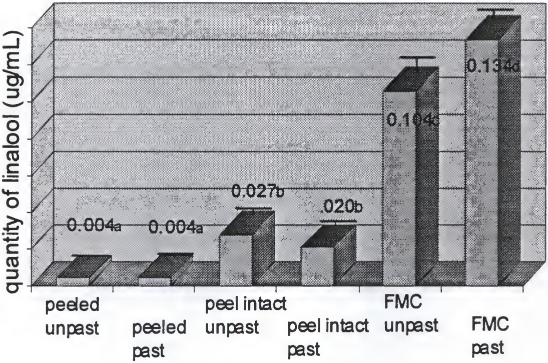
The single head FMC juice extractor macerates peel and ruptures many, if not most, of the oil glands found on the outer surface of the peel. The oil level in peel macerated juice was 0.018% or 225% greater than juice from hand reamed fruit (0.008%) and 300% greater than fruit peeled prior to juicing (0.006%).

Linalool Levels

Juices extracted with the peel macerating extractor contained the greatest quantities of linalool (unpasteurized 0.104 µg/mL and pasteurized 0.134 µg/mL). Samples peeled prior to juicing contained the lowest concentration (unpasteurized and pasteurized 0.004 µg/mL, Figure 7-1). Peel macerated juice contained 500% more linalool than juice from hand reamed fruit and 1000% more linalool than fruit peeled prior to juicing.

Heat Treatment Effect

Effect of heat treatment was inconclusive. While each hand squeezed, unpasteurized sample had slightly more linalool than the corresponding heat treated juice, the difference between each pair was not significant ($p<.05$). FMC extracted juice showed a different trend where pasteurized juice contained a significantly larger quantity of linalool than the unpasteurized (Figure 7-1).



Comparison with Previous Studies

As results from our study indicated, oil from orange peel appeared to be responsible for higher linalool concentrations in the study conducted by Nisperos-Carriedo and Shaw (1990) and Marin et al. (1992b). There was more linalool in aseptic packaged, reconstituted from concentrate juices, and in frozen concentrate juices. Peel oil is commonly added to "from concentrate" products to replenish lost aroma volatiles and improve flavor. Marin et al. (1992) concluded linalool was absent or barely detected in hand squeezed, unpasteurized juice, but was an important aroma contributor to pasteurized, mechanically squeezed orange juice. Mechanically squeezed juice was prepared with an FMC extractor (FMC, Lakeland, FL) that shreds peel and introduces oil into juice (Nagy, 1998).

Sensory

Results from DA are summarized in Table 7-2. Panelists found no difference between samples in green, fresh orange, musty-terpene, or sweet. Differences were found only between unpeeled and peeled hand squeezed juice. Peeled hand squeezed juice was significantly greater in cooked and sour than unpeeled hand squeezed juice. It was also significantly lower in fruity-floral ($p< .05$). FMC extracted juice did not differ significantly from the other juices.

Table 7-1 Average Descriptive Analysis Values for Unpasteurized juice.

	GREEN	FRESH ORANGE FLAVOR	FRUITY FLORAL	MUSTY TERPENE	COOKED
Peeled, Hand Squeezed	3.6^{a}	8.6^{a}	6.7^{a}	3.9^{a}	3.4^{a}
Peel Intact, Hand Squeezed	2.7^{a}	9.6^{a}	9.4^{b}	1.9^{a}	1.3^{b}
Peel Intact, FMC Extracted	3.4^{a}	9.4^{a}	$8.2^{a,b}$	3.3^{a}	$2^{a,b}$

Conclusion

The major source of linalool in orange juice was peel oil. Juice vesicle cytoplasm accounts for only a small portion. The effect of heat treatment was inconclusive and requires further investigation. Juices with higher levels of linalool (in peeled intact extraction) were perceived as having a significant greater floral fruity (a positive flavor attribute) and less of a cooked flavor (a negative flavor attribute)

CHAPTER 8

SUMMARY

Flavor research often requires a multifaceted approach. In the first few studies samples were determined to possess aroma and flavor differences (fresh, cooked, aged, cultivar specific) by sensory panel and juice samples were grouped differently through ENose evaluation. Volatile components were then extracted by SPME and pentane:diethyl ether and analyzed by GC, and MS. Finally sensory characteristics of extracted components were characterized and quantified by GCO and by sensory panel.

Technological advances in electronic sensing of volatile components by electronic nose instruments indicated the potential for replacing time consuming and laborious sensory panels in some cases. Research presented in Chapter 2 indicated a metal oxide sensor electronic nose was successful in distinguishing between grapefruit and orange juices, and between grapefruit juices of different cultivars. Organic polymer sensor electronic noses exhibited some ability to distinguish between orange juices exposed to differing heat treatments. In Chapter 4, statistical analysis of organic polymer sensor data from aged orange juices showed a similar trend to statistics from sensory panel analysis of the identical juices, and to statistics from headspace GC analysis of the identical juices. However, identities of volatiles that induced electronic nose sensor response were unknown. All components in headspace seemingly had the potential for producing a sensor response regardless of their importance in flavor. This was a major weakness in ENose technology. The development of sensors specific to flavor volatiles that impact food quality would be an improvement. In a preliminary step with Valencia juices, trained descriptive panelists found significant differences between unpasteurized and heat abused Valencia juices. Unpasteurized juice had a significantly higher score in fresh orange flavor, and a significantly lower score in cooked aroma than the heat abused juice.

Two techniques for volatile extraction were utilized: Solid Phase Microextraction and liquid-liquid extraction. Both techniques had advantages and disadvantages, and each lead to different conclusions about aroma volatile activities. SPME was easier to use, and faster than conducting liquid extractions. It was also very sensitive to experimental conditions. SPME was more efficient at extracting components in the terpene region. Pentane:diethyl ether extracted a wide variety of components and was not as sensitive to time or temperature of extraction as SPME. Pentane:diethyl ether was a laborious liquid extraction method. Both pentane and ether are highly volatile and care was taken to ensure these chemicals were kept cold.

Osme GCO measured aroma activities of volatiles. Components were identified by GC/MS as described Chapter 5. Identities of all important volatile components was not determined. Some had little or no corresponding GC peaks. Only volatile compounds that were extracted were evaluated, therefore important non-volatiles were not evaluated.

Recombination of aroma components deemed important by GCO in unpasteurized and heat abused orange juices were partially successful. Although the flavor of a model solution with aroma components added was determined not significantly different from unpasteurized orange juice flavor, another model solution with added components believed responsible for cooked flavor in orange juice was not significantly different from an unpasteurized orange juice either. Additional components are important in cooked flavor and they may be nonvolatile.

This research has demonstrated critical steps in flavor research: sample screening (ENose, taste panel), volatile extraction (SPME, liquid extraction), chemical analysis (GCO, GC, GC/MS), and sensory confirmation (GCO, sensory panel). Refinement of tools and techniques required at any of these steps would result in an advancement of flavor science.

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BIOGRAPHICAL SKETCH

Russell Albert Bazemore was born September 9, 1961 in the first Capital of the old South, Montgomery, AL. He was the fifth child of Wilbur and Edwina Bazemore. Russell attended Rolling Hills Elementary, Davis Hills Middle, and J.O. Johnson High School. Russell first enrolled at Huntingdon College, in Montgomery, AL, a small Methodist school. There he discovered his interest in chemistry under the supervision of Dr. Ward Knockemus and his desire for a diverse university free of religious affiliation. Russell transferred to Northeastern University (Boston) in September, 1981. After one year in New England, Russell transferred to the University of Alabama in Tuscaloosa for his final year of undergraduate studies. Following graduation in May 1983, Russell enrolled in post-baccalaureate studies at the University of Alabama before entering the U.S. Navy through Aviation Officer Candidate School in Pensacola, FL.

Russell was commissioned an Ensign on November 2, 1984. In eight years of military service he flew, sailed, and learned to look beyond the forecastle. Following an assignment as military advisor to the El Salvadorian Navy, Russell returned to graduate school at Oregon State University in June, 1992. Under the guidance of Dr. Mina McDaniel, he graduated in May, 1995 with an M.S. in Food Science and Technology. Upon graduation he began Ph.D. work with Dr. Russell Rouseff at the University of Florida where he plans to graduate December 19, 1998.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Professor of Food Science and Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Associate Scientist in Chemistry

This dissertation was submitted to the Graduate Faculty of the College of Education and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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